Biological Control of Postharvest Diseases of Fruits

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■ **Abstract**   Losses from postharvest fruit diseases range from 1 to 20 percent in the United States, depending on the commodity. The application of fungicides to fruits after harvest to reduce decay has been increasingly curtailed by the development of pathogen resistance to many key fungicides, the lack of replacement fungicides, negative public perception regarding the safety of pesticides and consequent restrictions on fungicide use. Biological control of postharvest diseases (BCPD) has emerged as an effective alternative. Because wound-invading necrotrophic pathogens are vulnerable to biocontrol, antagonists can be applied directly to the targeted area (fruit wounds), and a single application using existing delivery systems (drenches, line sprayers, on-line dips) can significantly reduce fruit decays. The pioneering biocontrol products BioSave and Aspire were registered by EPA in 1995 for control of postharvest rots of pome and citrus fruit, respectively, and are commercially available. The limitations of these biocontrol products can be addressed by enhancing biocontrol through manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of the biocontrol mechanisms, manipulation of formulations, and integration of biocontrol with other alternative methods that alone do not provide adequate protection but in combination with biocontrol provide additive or synergistic effects.

INTRODUCTION

The battle against postharvest decays of fruits and vegetables has been fought for decades but has not yet been won. Even the average consumer, who shops for quality fresh fruits and vegetables and must often discard spoiled produce, recognizes the persistent problem of postharvest decay. Although the development of modern fungicides and improved storage technologies in the 1960s and 1970s have greatly extended the shelf life of fruit after harvest, postharvest losses vary from an estimated 5 percent to more than 20 percent in the United States, depending on the commodity (24), and can be as high as 50 percent in developing countries (52). Postharvest losses have been reduced mainly through postharvest fungicides

(52, 53) and, to a lesser degree, through postharvest management practices to reduce inoculum or effective management of the cold chain system (keeping produce at low temperatures, which greatly reduce pathogen growth, from harvest to retail). However, postharvest use of fungicides has been increasingly curtailed by the development of pathogen resistance to many key fungicides (74, 137, 148, 180), lack of replacement fungicides, and public perception that pesticides are harmful to human health and the environment. This negative perception has promoted governmental policies restricting use of fungicides (73, 143). Thus, alternative methods to control postharvest diseases are urgently needed (35, 60, 157, 158, 165, 177, 183). Over the past 15 years, biological control has emerged as an effective strategy to combat major postharvest decays of fruits (82, 86, 112, 186). Compared to the long-standing interest in biological control of soilborne pathogens (182), research into biological control of postharvest decays (BCPD) is in its infancy. Nevertheless, progress has been substantial; the first commercial products have been registered in the United States by the U.S. Environmental Protection Agency (EPA) and are sold under the names BioSave100 and 110 and Aspire. In other countries such as South Africa, biocontrol products for the control of fruit diseases are registered [National Department of Agriculture Fertilizer, Farm Feeds, Agricultural and Stock Remedies (Act 36 of 1947)], and sold as Avogreen and YieldPlus.

Over two dozen programs worldwide are currently under way to develop BCPD of fruits, encompassing new approaches and methods to fit the postharvest system. General protocols for efficient discovery, scale-up, and pilot testing, necessary for rapid progress in developing biocontrol (37) have been developed and are driving this burgeoning area of research. In Europe, a multinational project is operating under the auspices of the European Commission (http://www.biopostharvest.org/). Commercial products are in the advanced stages of development and should reach European markets within the next year or two. Within governmental and industrial circles, there is firm commitment to accelerate development of new alternative strategies for the control of postharvest diseases.

This review describes the uniqueness and key strategies underlying the development of biological control of postharvest diseases of fruits, possible approaches to increase the spectrum of activity, commercial successes, and prospects for the future.

UNIQUENESS OF THE POSTHARVEST SYSTEM

Progress in BCPD, especially in the postharvest application of antagonists, may be attributed to the uniqueness and relative simplicity of the postharvest system. Wounds made during harvesting and fruit handling can be protected from wound-invading pathogens with a single postharvest application of the antagonist directly to wounds, using existing delivery systems (drenches, on-line sprayers, on-line dips). Once harvested, fruits are placed in cold storage for various periods of time

ranging from a few days to months, depending on the commodity. Environmental conditions, such as temperature and relative humidity, can be managed to favor antagonist survival. Furthermore, biotic interference is minimal so antagonists encounter minimal competition from indigenous microorganisms. Consequently, biocontrol of postharvest diseases tends to be more consistent than biocontrol under field conditions, and the occasional variation in performance usually can be traced to nonstandard procedures or conditions. The levels of decay acceptable in postharvest systems are generally below 5 percent, a standard that is often achieved with a single application of the biocontrol agents. Given the high retail value of fresh fruits, the application of high concentrations of the antagonists to fruit surfaces is economically viable, whereas under field conditions this usage might not be cost effective.

The short period between harvesting and placing fruit in storage, from less than a day to a few days, requires rapid antagonist action. Once fruit is placed in cold storage, metabolic rates of the host and associated microflora will decline depending on the temperature regime selected. The search for antagonists to control postharvest wound invading pathogens should be narrowed to rapid colonizers of the wound site that can still be metabolically active at low storage temperatures.

The ecology of the targeted pathogen must be understood in developing a biological control strategy. Pathogens that tolerate environmental stress often have few competitors, since few species can exist under such conditions. For example, the opportunistic pathogen *Botrytis cinerea* may be a poor competitor (14) in comparison to *Penicillium* spp., which often produce secondary metabolites that inhibit competitors. Stress-tolerant and competitive species would therefore require biocontrol strategies different from those of ruderal species (14), which depend upon physical adaptations to limited environmental resources or carrying-capacity environment, and are more stable and permanent members of the community (9).

Some postharvest rots result from preharvest latent infections, especially in tropical and subtropical regions where environmental conditions in the field are particularly conducive to fruit infection. Controlling rots resulting from preharvest latent infections with postharvest treatments is difficult. Nevertheless, successful control of latent infections by postharvest applications has been reported (103, 106, 111). *Bacillus* spp., which are dominant colonizers of the phylloplane and fructoplane, can control latent infections by *Colletotrichum* spp. on mango and avocado (43, 44, 108). Rapid colonization by the antagonist may not be necessary for the control of rot resulting from latent infections. In the biocontrol of anthracnose of avocado, *Bacillus subtilis*, applied as a dip or a wax formulation removes nutrients from the immediate surrounding of the appresorium of *Colletotrichum gleosporioides* to ensure maintenance of the dormancy, as demonstrated by Korsten et al. (111).

The main strategy used to suppress postharvest fruit decay is the postharvest application of antagonists to prevent pathogens from infecting fruit wounds after harvest, but postharvest decay can also be suppressed by field application of biocontrol agents (16, 110, 114, 173). Field applications have been successful against

anthracnose caused by *Colletotrichum* sp. on mango and avocado (108, 111), and to a lesser extent against *Pezicula malicorticis* on apples (114), using multiple applications of the antagonist in the orchard. Although sometimes useful in control postharvest decays from wound infection (16, 172), preharvest applications of antagonists may serve only as a supplement to their postharvest application. To control postharvest decays originating from latent infection in the orchard, the application of antagonists must be repeated. Korsten (107) showed that three sprays with *B. subtilis* controlled anthracnose of avocado as well as three copper sprays, but the volume of biocontrol product required to spray the trees was large. In addition, continuous application of *B. subtilis* over a four-year period resulted in a gradual build-up of the antagonist, reduction of the level of pathogen inoculum, and more effective control.

PATHOGENS VULNERABLE TO BIOCONTROL

The most successful biocontrol systems against plant pathogens, *Agrobacterium radiobacter* strain K-84 (currently strain K-1026) against crown gall caused by *A. tumefaciens*, and *Phlebia gigantea* against annosus root rot of pine, are based on a single application of the biocontrol agents directly to the wounds (uncolonized in the case of *Phlebia*), the site of pathogen entry (105, 145). Wounds are "an ideal environment for antagonists because they provide a moist, nutrient-rich substrate with initially no competition from other microorganisms" (18). However, to sustain such a dominant position in wounds requires a pre-emptive colonization strategy by the antagonist to ensure that the environment is "managed" for as long as possible so that it remains unfavorable for other microorganisms. Natural antagonists can be classified with the *r-K* gradient of population strategies within microbial communities. The properties of an effective biocontrol agent will therefore depend on the setting in which it is intended to function (14). The ability to rapidly colonize can be ascribed to the typical *r* strategist. *Pseudomonas* spp. and many yeasts are typical *r*-strategists, which grow rapidly, dominate, and colonize the new niche where resources are temporarily abundant. Biological agents that are *r*-strategists can be compared to a protectant fungicide that is in place before onset of the pathogen infection cycle (14). Where a pathogen has already invaded the plant host, a more competitive species with a high tolerance to abiotic stress will be required.

Plant pathogens are typically spread across the *r-K* range of characteristics. Brown rot (caused by *Monilinia fructicola*) of peach, which can originate from both latent infections in the orchard and wounds made after harvest, would thus require both *r* and *K* strategists applied in both preharvest and postharvest settings to achieve optimal control (139, 140). Wound-invading necrotrophic fungi such as *Penicillium expansum* and *B. cinerea*, which cause postharvest blue mold and gray mold on apples and pears, respectively, require nutrients for germination and initiation of the pathogenic process. This requirement makes them suitable for biocontrol through nutrient competition. The pome fruit system is well suited for

biocontrol, and many successful attempts have been reported from various labora-
tories (23, 26, 30, 32, 34, 55, 59, 80, 90, 101, 118, 123, 142, 147, 150, 152, 162, 178,
191). In addition, decay was reduced significantly after preharvest orchard applica-
tion of antagonists, which allowed antagonist populations to become established on
fruit surfaces before anticipated wounding during harvest (16, 172). A biocontrol
system for reducing decays of citrus is also promising (6, 19, 27, 47, 48, 75, 125,
153, 155, 184), especially in locations where most decays are caused by wound-
invading *Penicillium italicum* and *P. digitatum*, which cause blue mold and green
mold, respectively. However, tests in citrus packinghouses indicated that biocon-
trol alone cannot provide adequate control and must be combined with diluted
fungicides or other methods of control (47, 48). Other successful attempts have
been made to control wound-originating postharvest decays on cherry (31, 179;
L. Grant, EcoScience Corp., unpublished information), grape (15), tomato (28),
kiwi fruit (33), strawberry (72, 78), and banana (42, 103).

The potential for biological control of postharvest diseases of vegetables has
been reviewed recently (91).

SEARCH FOR THE ANTAGONIST

Sources and Isolation of the Antagonist

The fruit surface is an excellent source of naturally occurring antagonists against
postharvest fruit decay. For example, searching for antagonists on healthy apples
in the orchard and storage, "a place where a disease can be expected but it does
not occur" (10), resulted in the isolation of many ecologically fit bacterial and
yeast antagonists effective against postharvest decays of apple (80, 83). Similar
isolations from other fruits also yielded effective antagonists against postharvest
fruit decay pathogens (3, 6, 19, 72, 75, 104, 118, 141, 175, 184, 195). Isolation of
the antagonists can be improved by using fruit from unmanaged orchards (59, 85),
where natural populations have not been disturbed by chemical usage, and the
pool of potential antagonists is greater than in a chemically managed orchard
(160). A variety of enrichment procedures have been used that favor isolation of
microorganisms growing efficiently on the substrate, which occurs at the infection
site (wound) that must be protected. These include isolation from natural cracks
on the fruit surface (W.J. Janisiewicz, unpublished); agar plates containing apple
juice that were seeded with fruit washings (83); fruit wounds treated with fruit
washings and incubated for several days (187); freshly made wounds on apples in
the orchard that were exposed to colonization by fruit-associated microbiota from
one to four weeks before harvest (85); and from an apple juice culture resulting
from seeding diluted apple juice with the orchard-colonized wounds and repeated
reinoculation to fresh apple juice.

The fructoplane has provided the most abundant and most desirable source for
isolating antagonists against postharvest fruit pathogens. However, the antagonists
may also come from other closely related or unrelated sources. The phylloplane

has also been a good source of antagonists, as it may share part of the resident microflora of fruits as well as contain other microorganisms dislodged from the fruit (59, 80, 109, 110, 163, 196). Screening collections of yeast (64) or starter cultures used in the food industry (138) may also yield effective antagonists. Soil also may be an abundant and diverse source of antagonists. *Bacillus subtilis* B3, a contaminant found during routine isolation of fungi from peach roots and identified for its inhibition of *M. fructicola* on agar plates (J. Barrat, personal communication), is a very effective strain for biocontrol of brown rot of peach (139). B3 was the first strain studied extensively for biocontrol of postharvest diseases, and early successes in biocontrol of brown rot on peach with this strain (139) stimulated interest in biocontrol of postharvest diseases of fruits in general.

Selection Criteria

Cook stated, "a serious shortcoming of microbial biocontrol as currently tested and studied is that too few strains are examined" (36). To some extent, this shortcoming is also true for BCPD. Although screening of large numbers of organisms has been reported, only recently has more effort been devoted to testing the biocontrol potential of various strains of the same species of yeast and bacterial antagonists (95, 149).

For biocontrol to be successful, the conditions that favor a potential antagonist should be the same or similar to those that favor the pathogen. The best antagonists perform well over the full spectrum of conditions conducive to pathogen development. Strains of an antagonist species can be compared for effectiveness in controlling fruit decay, and for phenotypic characteristics that are useful in determining their commercial potential. For example, differentiation criteria for decay control on apple can include the biological control efficacy of the strains, spectrum of activity (pathogens to be tested, cultivar range, fruit maturity stages), ability to colonize wounded and sound fruit surfaces under various conditions, utilization of substrates occurring in fruits, or growth at cold storage temperatures and at 37°C (human body temperature). Biocontrol agents are more acceptable if they can be applied together with current practices, and information on the compatibility of the biocontrol agents with chemicals used in the postharvest system, e.g., antioxidant diphenylamine (DPA) used for control of superficial scald of apple, a physiological disorder, or flotation salts used to increase buoyancy of pears during handling in water, should be developed. Additional criteria may include resistance to environmental stress in the orchard application of biocontrol agents (16, 77, 114, 172, 174), and pathogenicity of the antagonists to fruits, since strains of some antagonists with good biocontrol potential, e.g., *Aureobasidum pullulans*, can cause minor decay and russeting on some fruits (51, 124, 146).

Since the antagonists are applied to consumable products (produce), they must meet strict requirements for human safety. The initial determination of the antagonist's safety is based on the available literature and should be made early in the program, before committing time and resources into further research. The final

determination is made during commerical development of the product, and is based on an elaborate and costly toxicological profile.

Genetic and physiological niche requirements can vary among strains within a species, and intraspecies variation is poorly understood. Some species are isolated repeatedly from the same type of fruit at various geographical locations. For example, of 13 yeast species reported to be residents of apple (40), 7 (30, 32, 59, 76, 93, 95, 114, 118, 127, 147, 192) were isolated and reported to have strong antagonistic activity against postharvest decays of pome fruits by laboratories at different geographical locations. The biocontrol potential of strains of the same species from different locations may well vary, and this subject warrants in-depth investigation. In fact, the biocontrol potential and physiological and genetic diversity of the yeast *Metchnikowia pulcherrima* appears to be great even at a single geographical location, and provides a wide variety of organisms with desirable biocontrol traits (95).

Thus, in developing biocontrol, the key requirements for successful commercialization of an antagonist must be well defined, and strain searches should continue until adequate strains are found that meet all requirements. Some of those requirements are intuitive (186), but others involving mass production, formulation, application, and distribution require a more intimate knowledge of commercialization of microbial pesticides (66, 102, 136, 166). In this regard, a significant benefit can be derived from developing, early in a program, a liaison between a scientist working on developing a biocontrol system and the industry that can commercialize the biocontrol agent (166).

MECHANISMS OF BIOCONTROL

The mechanisms of BCPD are poorly understood, mainly because relatively few attempts have been made and appropriate methods to study microbial interactions in wounds of fruit are lacking. Various mechanisms have been described, including antibiosis, production of lytic enzymes, parasitism, induced resistance, and competition for limiting nutrients and space. Often, more than one mechanism was implicated, but in no case has a sole mechanism been found responsible for biological control.

The antibiotics iturin, produced by *B. subtilis* B-3 (70), and pyrrolnitrin, produced by *Pseudomonas cepacia* LT-4-12W (94), reduced in vitro growth and conidia germination of the stone fruit pathogen *M. fructicola*, and pome fruit pathogens *P. expansum* and *B. cinerea*, respectively. Both strains controlled fruit decays caused by the respective pathogens (94, 139), and strain LT-4-12W also controlled various decays on citrus (155) and stone fruits (156). These fruit decays were also controlled by applications of the respective antibiotics alone (79, 138). However, the significance of the antibiotics in these biocontrol situations is not clear, since strain LT-4-12W still provided substantial control of blue mold decay on oranges inoculated with laboratory-derived mutants of *P. itallicum* resistant to pyrrolnitrin (J.L. Smilanick, personal communication). No analogous tests were conducted with iturin. The mechanism(s) of biocontrol of *Pseudomonas syringae* strains

ESC-10 and ESC-11 (formerly known as L-59-66) used in BioSave products has not been elucidated. Bull et al. (22) showed that on some media both strains can produce syringomycin E, which is inhibitory to a variety of fungi, and that the purified compound can control green mold of lemons. However, the role of syringomycin E in biocontrol is in doubt because efforts to isolate this compound from fruit wounds treated with the antagonist have been unsuccessful (C.T. Bull, personal communication), and rapid growth and colonization of the wounds was important for biocontrol. This suggests that competition for nutrients and space may have played a major role (21).

The attachment of antagonists to pathogen hyphae has been suggested as an important factor in competition for nutrients between the antagonist *Enterobacter cloacae* and *Rhizopus stolonifer* on peach (193), and between the antagonistic yeast *Pichia guilliermondii* and *P. italicum* on citrus fruit (8), or in the lysis of *B. cinerea* by β-1,3 glucanase produced by *P. guilliermondii* on apple (190). Given the very high density (10^{10} cfu/ml) of *E. cloacae* needed to inhibit conidia germination and decay, exclusion may also play an important role in biocontrol. The production of ammonia by this antagonist has been reported as a mechanism of biocontrol in another system (65) and should be given consideration in the fruit system as well. To determine the significance of attachment and β-1,3 glucanase in biocontrol by *P. guilliermondii*, confirmatory tests in fruit wounds are needed. Filonow (62, 63) showed that the apple volatile, butyl acetate, stimulated adhesion to membrane filters and germination of conidia of *B. cinerea* and increased apple decay. The antagonists *Cryptococcus laurentii* and *Sporobolomyces roseus* but not the baker's yeast *Saccharomyces cerevisiae* used butyl acetate as a food source and reduced these stimulatory effects in vitro. These effects, however, have not been shown in apple wounds owing to technical difficulties in conducting this type of experiment. In addition, studies with various sugars occurring in apple showed greater uptake and utilization of ^{14}C fructose, and stronger inhibition of conidia germination in diluted apple juice by the antagonists other than *Saccharomyces cerevisiae*, suggesting that competition for nutrients may play an important role (61).

Jijakli & Lepoivre (100), and Gravese et al. (69) have shown that the yeast *Pichia anomala* strain K, effective in the control of gray mold of apple, increased production of exo-β-1,3-gluconase threefold in the presence of cell wall preparations of *B. cinerea* in apple wounds, reducing lesion size by more than half compared to the antagonist alone. This strengthens the hypothesis that exo-β-1,3-gluconase is involved in the biocontrol of gray mold by this antagonist. Higher β-1,3-glucanase and chitinase activity was also detected in apple wounds treated with strains of an antagonist, *A. pullulans*, effective in controlling various decays on apple, table grape, and other fruits (25, 76). The increased amount of these enzymes was attributed to higher production by the antagonist and to the induction of the enzymes in the fruit itself. The source of these enzymes and their significance in biocontrol warrant further investigation. Further evidence for the significance of these enzymes in biological control could come from studies evaluating disease suppression by mutants with a disrupted β-1,3-glucanase gene. *A. pullulans* may also

produce antibiotic aurebasidins, whose role in biocontrol should be considered
(171). *Erwinia herbicola* strains B66 and B90, which controlled blue and gray
mold of apple, demonstrated taxis to the conidia and germ tubes, inhibited coni-
dia germination, and lysed germ tubes of *B. cinerea* and *P. expansum* in diluted
apple juice (20, 162). These interactions were not as apparent in undiluted apple
juice, suggesting that competition for nutrients may be important in this system.
The yeast *Candida famata* reduced green mold decay (caused by *P. digitatum*) on
oranges and increased the phytoalexins scoparone and scopoletin 12-fold in fruit
wounds after four days when inoculated alone (7). However, the significance of
phytoalexins in this biocontrol is not clear because of their relatively slow pro-
duction. Electron microscopic observations indicate rapid colonization and partial
lysis of the pathogen's hyphae by the antagonist.

In only a few of these examples has work continued beyond the initial reports
to fully explain the biocontrol mechanisms, mainly because developed methods
to study mechanisms of biocontrol in BCPD, in particular competition for nutri-
ents, are lacking. In almost all cases, nutrient competition was reported to play
a significant role, but it is difficult to separate from other mechanisms. However,
recent reports on developing biological sensors (121) and using natural substrates
in in vitro cylinder-well tests for studying antagonist-pathogen interactions (96)
may be useful in studies evaluating microbial competition for nutrients in fruit. A
biological sensor, composed of a nutrient-responsive promoter fused to a reporter
gene, could be used to assess the spatial distribution and availability of nutrients
in fruit wounds at a critical time for pathogen. Reporter genes encoding the Green
Fluoresent Protein (GFP) or ice nucleation protein (132) are especially useful for
studies evaluating gene expression by bacterial antagonists on and in plant tissues.
In cylinder-well tests, the antagonist and pathogen are separated by a membrane
and immersed in fruit juice, which can flow through the membrane. After an in-
cubation period, the pathogen can be removed and evaluated for viability, ability
to infect fruit, or susceptibility to other mechanisms of biocontrol.

Applied microbial ecology was an initial driving force in the commercial de-
velopment of BCPD of fruits, but further progress in improving biocontrol will
largely depend on the basic understanding of the mechanism(s) of biocontrol.

ENHANCEMENT OF BIOCONTROL

In comparison to the field environment, post-harvest environments are well defined;
abiotic and biotic factors can be determined with relative ease and manipulated
to an antagonist's advantage. Although the mechanism(s) of biocontrol have not
yet been fully explained and, to date, there have been only a few attempts to ex-
ploit these mechanisms to improve postharvest biocontrol (23, 97), reports on the
mechanism of BCPD suggest that competition for nutrients and space plays a ma-
jor role in most cases (23, 31, 46, 61, 64, 85, 96, 110, 181, 190). Rapid colonization
of fruit wounds by the antagonists is critical for decay control, and manipulations

leading to improved colonization enhance biocontrol (97, 130). Within microbial communities, interactions are density dependent, and more than one type of interaction can occur at any one time, depending on the growth phase of different microorganisms, population density, and species diversity. Three different types of interactions, competition for nutrients, competition for space, and inhibition by secondary metabolites, were observed with preharvest sprays of *B. subtilis* to control *C. gleosporioides* on avocado (110). The main approaches used to improve BCPD are (*a*) manipulation of the environment, (*b*) use of mixed cultures of antagonists, (*c*) physiological and genetic manipulation of antagonists, (*d*) combining field and postharvest applications, (*e*) manipulation of formulations, and (*f*) integration with other methods.

Manipulation of the Environment

It is possible to manipulate the physical and chemical environment to the advantage of antagonists in storage, but fruit quality must be maintained. Temperature, humidity, and often gas compositions are predetermined to maintain fruit quality, and the antagonists must be well adapted to these conditions (44, 178). Fruits are often treated and/or handled in a water suspension before, during, and after storage, which provides an excellent opportunity to modify the environment. Nitrogen is likely to be a limiting nutrient in the carbon-rich environment of apple and pear wounds. The addition of L-asparagine and L-proline enhanced populations of the antagonist *P. syringae* in wounds of mature apple fruit more than tenfold during the critical first 24 h at room temperature and the first month of storage at 1°C, resulting in reduction of blue mold decay from as much as 50% to 0% (97). These nutrients were selected after in vitro screening of various nitrogenous compounds for preferential stimulation of the antagonist's growth over mycelial growth and conidial germination of the pathogen *P. expansum*. The addition of a nutrient analog 2-deoxy-D-glucose, which is taken up by the pathogen but is not metabolized, thus inhibiting pathogen growth and giving advantage to antagonists *P. syringae*, *S. roseus*, and *Candida saitoana*, improved biocontrol of decays of apple and citrus, respectively (58, 84). The addition of siderophores may reduce apple decay by sequestering iron required for germination of some postharvest pathogens (23). It may also stimulate production of the antagonist's siderophores by creating an iron-deficient environment at the wound site. Despite its great potential for improving biocontrol, manipulation of the chemical environment has not been widely exploited. This may be attributable to limited knowledge of the mechanisms of biocontrol. If nutrients limiting growth of pathogens or antagonists were known, then limiting nutrients or substances could be manipulated to stimulated antagonist populations and/or biocontrol mechanisms.

Applying Mixed Cultures

It has been difficult to select individual strains with a broad spectrum of activity against major and minor pathogens that are effective when used on fruits at various

maturity levels. Mixtures of compatible strains may be needed to provide the necessary spectrum of activity. Components of the microbial community that contain the desired antagonistic attributes might be reconstructed by selecting them from fruit wounds, growing them in culture, and applying them to fruit surfaces as a mixture. In this case, the antagonistic action will result not from an activity of one species but rather from the action of a community of microorganisms that suppress a target pathogen through different mechanisms of action. However, such community reconstruction may be very challenging, as some microrganisms are incapable of independent growth under common cultural conditions (5). A better understanding of microbial communities on fruit is needed to take full advantage of this resource.

The application of antagonist mixtures has reduced variability and improved efficacy of biocontrol in many systems, some of which include pathogens that infect fruit (67, 71, 120, 135, 144, 151). With the exception of work on biocontrol of anthurium blight, where microbial communities of guttation fluids were screened for biocontrol activity (68), no special criterion was used to select antagonists for mixtures. On apple, a broader spectrum of pathogens was controlled (81), and less total biomass of the antagonist was needed to control decay when antagonists were applied in mixtures instead of individually (85, 87, 114). The mixtures were composed of antagonists paired at random (81, 114), or after screening for minimum mutual niche overlap in the utilization of nutrients, assessed on BIOLOG standard plates (85) or on customized BIOLOG plates containing nutrients occurring at the wound site (W.J. Janisiewicz, unpublished data). The BIOLOG approach reduced selection of strains competing for the same nutrients, but did not eliminate potential negative interactions that may result from the production of secondary metabolites (114). Thus, to determine further compatibility of the strains selected for a mixture, it is important to conduct coexistence studies using the De Wit displacement series (189) in fruit wounds (85). Antagonists selected for mixtures may also be obtained from microbial succession at the wound site. First, the sequence in colonizing freshly made apple fruit wounds in the orchard just before harvest must be determined and then the organisms for the mixtures selected from the succeeding organisms. A large number of such organisms were isolated and screened for antagonistic activity directly on apple fruit. The largest number of organisms antagonistic to *P. expansum* were found in wounds sampled closest to harvest (85). The isolated antagonists were ecologically suited to the chemical environment of the fruit, and selection of strains suited for the stable physical environment in storage was not difficult. We can also explore relevant examples of microbial interaction in food products. For example, the succession of yeasts in the apple cider–making process may closely resemble those at the wound site where apple juice is the main substrate (13, 17). After exhausting limiting nutrient(s) by one organism, another organism, originally less competitive but not requiring or able to synthesize the limiting nutrient, may take over colonization of the wound, further depleting nutrients utilized by the pathogen. To predict competitive interactions between antagonists in a mixture and between a

pathogen and the antagonists, the nutrient composition at the wound site must be known.

The benefits of using an antagonist mixture are clear, but implementation of this approach requires approval from the industry producing biocontrol agents, because it entails a doubling of the cost to commercialize the antagonist mixture as compared to a single antagonist. The economic viability of this approach is favored if mixtures include at least one antagonist that has already been commercialized (W.J. Janisiewicz, unpublished findings).

Physiological and Genetic Manipulations

Physiological manipulation of antagonists has been focused on improving their ecological fitness, which is particularly important in orchard applications where environmental conditions fluctuate widely. Teixidó et al. (173) reported that populations of low water activity (a_w) tolerant cells of the antagonist *Candida sake* obtained from growth on media modified for low (a_w) and applied to apples 2 days prior to harvest, increased until harvest time, whereas those from nonmodified media remained relatively unchanged. Population increases of both types of cells were similar during four months of cold storage of the fruit. Although no significant difference in biocontrol of blue mold on apples was observed between the two types of cells, this study showed the potential of using this approach to increase antagonist populations, which could enhance biocontrol in other systems where antagonist populations declined after orchard application (16).

Physiological manipulation may also be used to enhance mechanisms of biocontrol. This has been demonstrated for soil antagonists, where the addition of zinc increased production of the antibiotic, phenazine, which is a mechanism by which *Pseudomonas fluorescens* suppresses take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* (154). In a postharvest system, Calvente et al. (23) reported that iron sequestration by a siderophore, rhodotorulic acid, produced by the yeast *Rhodotorula glutinis*, is responsible for biocontrol of *P. expansum* on apples. The addition of the siderophore to the antagonist suspension further increased biocontrol of blue mold on apples, whereas the addition of iron reduced biocontrol. This study demonstrates that competition for iron can be an important biocontrol mechanism on fruit.

Genetic manipulation of antagonists to improve BCPD is a field in its infancy. Current efforts are focused on developing efficient transformation procedures for bacterial and yeast antagonists and inserting genes for tracking the antagonist in the environment rather than enhancing biocontrol (12, 133, 194). However, attempts to overexpress genes involved in biocontrol, e.g., lytic enzymes, or engineering strains with desired biocontrol traits (M. Wisniewski & W.J. Janisiewicz, unpublished results), may soon yield positive results.

Preharvest Applications

There are two distinct approaches in applying biocontrol agents in the field to control postharvest decays of fruits. In one, antagonists developed for postharvest

application are applied just before harvest. The intent is to precolonize the fruit surface with an antagonist immediately before harvest, so that wounds inflicted during harvest can be colonized by the antagonist prior to colonization by a pathogen (77). In the other approach, antagonists, selected for field application, are applied throughout most of the fruit development, to reduce latent infections that can originate as early as bloom time and cause fruit decay after harvest when the natural mechanisms of resistance have broken down. These two approaches are different from biological control of fruit decays in the field, which are beyond the scope of this review (50). In both approaches, many of the advantages of postharvest application of biocontrol agents discussed earlier are lost. However, both approaches have had some successes. The antagonistic yeasts *Cryptococcus infirmo-miniatus*, *C. laurentii*, and *R. glutinis*, applied to d'Anjou and Bosc pears in the field 3 weeks before harvest maintained high population densities through harvest and, in the best cases, reduced gray mold on Bosc pear from 13% to as little as 4%, and on d'Anjou pear from 7% to as little as 1% on fruit that were wounded after harvest and stored for 4 months at 0.5°C (16). Given such a high reduction in decay from just one preharvest application of the antagonist, it would be interesting to determine how much more protection can be achieved by an additional application after harvest, when postharvest fungicides are routinely applied. Populations of antagonists such as *A. pullulans*, *R. glutinis*, and *B. subtilis* adapted to variable environmental conditions, increased after application to apples in the orchard, and were maintained at relatively high densities on fruit in cold storage, except for *B. subtilis*, which declined (114). Mixtures of these antagonists controlled blue and gray molds and bull's-eye rot caused by *P. malicorticis* as effectively as the fungicide Euparen, and were more effective than the individual antagonists in tests on apples after harvest. Applying mixtures of these antagonists in the orchard may be useful for controlling postharvest decays, especially if applied early in the growing season, which may reduce latent infection caused by *Pezicula* spp. *C. sake* CPA-1 reduced blue mold by ~50% on wounded apples inoculated with the antagonist 2 days before harvest and then inoculated with *P. expansum* before placing in cold storage for 4 months (172). Although these procedures would be unlikely under commercial conditions, this work confirmed that wounds precolonized by the antagonist in the field are much more difficult to infect by the pathogen. Here too, it will be interesting to see how much more decay control can be achieved with an additional antagonist application after harvest.

The control of postharvest decays of strawberries has been very difficult, even with preharvest fungicidal applications. Field infections are considered to be the main source of fruit decay because *Botrytis* infections can occur from bloom to harvest and often develop into decay on mature fruits (98). Field applications of various antagonists including *Gliocladium roseum* (169), *Trichoderma hazianum* (113, 176), *B. subtilis*, and *B. licheniformis* (L. Korsten, unpublished data), yeast, and other bacteria (F. Takeda & W.J. Janisiewicz, unpublished data) from bloom until harvest have had variable success. Less variable results were obtained in controlling fruit decays in greenhouse strawberry culture, and antagonists on flowers and fruit remained at higher and more stable populations under controlled

greenhouse conditions (119; F. Takeda & W.J. Janisiewicz, unpublished data). The best control, however, was obtained with the application of pyrrolnitrin, a secondary metabolite from the biocontrol agent *P. cepacia*, on harvested fruit to control postharvest decays (170). Anecdotal evidence suggests that postharvest infection originating from wounds made by pickers, basket abrasions, and handling may be a major cause of strawberry decay (41; W.J. Janisiewicz, personal observation). Perhaps biocontrol efforts should be focused on the control of these kinds of infections. In this respect, there is a recent, promising report on the successful biocontrol of strawberry decay with postharvest application of *Candida reukaufii*, *C. pulcherrima*, and strains of Enterobacteriaceae isolated from strawberry fruits (72).

In the examples above, significant biocontrol of postharvest decays on the same kind of fruit was achieved with both field and postharvest applications. Combining field and postharvest application of biocontrol agents should lead to even more effective control of postharvest decays.

Manipulation of Formulations

Formulations have a profound effect on biocontrol agents and products, including shelf life, ability to grow and survive after application, effectiveness in disease control, ease of operation and application, and cost (66). Formulation of microorganisms for biocontrol of plant pathogens is undeveloped compared with other applications of microorganisms. Formulations of commercial products for postharvest applications remain mostly proprietary, but research in the public sector is recording progress on formulations for improving on the viability, efficacy, and shelf life of biocontrol agents. Certain freeze-drying protective agents and rehydration media enhanced the viability of the antagonist *Pantoea agglomerans* strain CPA-2, effective against blue mold and gray mold of pome fruits (38). The greatest protection from freeze-drying injury to the antagonist was provided by 5% trehalose, with the survival of over 60% of the cells, whereas 10% skim milk was the best rehydration medium, resulting in a 100% recovery of the freeze-dried bacterium. At high initial cell densities of the antagonist (10^{10} cfu/ml), sucrose was a very effective protectant, whereas rehydration media had no effect on recovery. Survival of cells of the antagonistic yeast *C. sake* was improved from 0.2% to 30–40%, by using freeze-drying protective media consisting of skim milk and other protectants, such as 10% lactose or glucose, and 10% fructose or sucrose. However, the shelf life of the product has been poor and needs additional improvement (1). The addition of xanthan gum to *A. pullulans* L47, applied to strawberries in the field from bloom to fruit at the green stage, improved survival of the antagonist and increased biocontrol of storage rot caused by *B. cinerea* (78).

Formulations can influence the survival and activity of biocontrol agents on fruit surfaces and in wounds. In some spore-forming bacteria such as *Bacillus* species,

breaking the endogenous dormancy of the formulated product may be a factor in the speed of action, which is crucial in postharvest biocontrol. Formulations that include wetters (humectants) to facilitate reabsorption of moisture from air may reduce this problem (102). Wetters not only make water spray stay on plants but, like oil carriers, they also enable organisms to reach otherwise inaccessible places such as depressions, stomata, and lenticels, thereby improving the chances of establishing antagonists for disease control. Oil carriers are expensive, but formulations containing oils can enhance the reliability of biological control agents (102). Ultra low volume (ULV) sprays give good cover in foliage canopies under suitable conditions and are relatively cost effective. Also worth exploration is the use of natural pigments such as melanins, which are nature's super sunscreen and also improve dessication tolerance and protect against hydrolytic enzymes. Research is needed to determine the value of each additive alone and also in the presence of other ingredients.

Improvements in formulations can result in substantial benefits to biological control, but research in this area has been limited. A more systematic approach to improve formulations is required and could be undertaken with knowledge of the biocontrol agent's mode of action and the windows for effective use, especially under field conditions. An understanding of agricultural use and market factors is also needed to improve biocontrol formulations. A shelf life of less than 6 months requires direct order service, whereas 0.5–2 years is adequate for conventional off-shelf sales. The biocontrol products BioSave100 and 110 have been formulated as frozen pellets and as a wettable powder. The frozen formulations require a cold chain and extensive field services to assure high quality of the product (L. Grant, EcoScience Corp., personal communication). The shelf-life limits of each biocontrol agent is probably controlled by its genetic composition. These limits might be extended both by evaluating more strains and by genetic engineering with genes affecting survival traits.

Integration with Other Methods

During the past few decades, many attempts have been made to develop nonfungicidal methods to control postharvest decays on various commodities. They include environmental modification such as storing commodities at temperatures suppressive to pathogen development, modifying relative humidity and the atmosphere, and treatment with hot air or water (60, 161, 164); inducing resistance by applying elicitors (54) or UV irradiation (45, 168); applying substances generally regarded as safe (GRAS) (35, 157–159); and sterilizing fruits and handling water with UV irradiation (134) or ozone (177). However, none of these methods, when used alone, provided satisfactory levels of decay control, although some appeared to be very useful when applied in combination with biological control, resulting in additive or even synergistic levels of decay control.

Applying a 2% solution of calcium chloride together with the yeast antagonist *Candida* spp. enhanced biocontrol of gray and blue molds on apples (128, 191), but calcium chloride solution alone did not reduce decays. However, application of 68 mM $CaCl_2$ to grapefruit reduced the incidence of green mold decay by 43%, and in combination with an antagonist *P. guilliermondi* strain US-7 by 97% (49). Pressure infiltration of 0.27 M calcium chloride into apples increased calcium content of the mesocarp threefold and reduced blue mold decay on Golden Delicious apples inoculated with *P. expansum* after 6 months in cold storage by as much as 50% (88). Combining calcium chloride pressure infiltration with the application of the antagonist *P. syringae* (isolate ESC-11 used in BioSave110) resulted in greater control than the individual treatments (88). Integrating these two treatments has the added benefit of increased Ca to alleviate physiological storage maladies such as bitter pit, and reduced amounts of both products to be used without compromising decay control.

Prestorage hot air treatment (38°C for 4 days) of apples reduced or eliminated blue mold decay caused by *P. expansum* and gray mold decay (60). Heat had eradicative activity on decay of apples inoculated with *P. expansum* 12 h before heating. Heat also improved biocontrol with heat-tolerant yeasts when applied to apples up to 24 h after inoculation with the pathogen (116). Combining the heat treatment with Ca infiltration and then applying the biocontrol agent *P. syringae* ESC-11 was most effective in reducing blue mold decay compared with individual or other treatment combinations, when mature fruit, after 6 months in storage at 1°C, were inoculated with *P. expansum* alone or in a mixture with the antagonist. The heat treatment provided little residual protection, but the residual protection provided by Ca and the antagonist added to the control by heat (117). When antagonists were applied to apple wounds before heat treatment, the heat reduced populations of *P. syringae* and increased populations of the two heat-tolerant yeasts more than tenfold (117). After removal from the heat and placement of fruits in cold storage, populations of *P. syringae* increased, but not to the original application level, and those of the yeasts continued to increase. The addition of the heat treatment improved control of blue mold for this and two other heat-tolerant yeast antagonists. Hot water (45–56°C) dip treatments are used commercially in citrus and mango packinghouses in South Africa to reduce postharvest decays. The efficacy of control could be enhanced by the addition of *Bacillus* spp. to the hot water dips (L. Korsten, unpublished data).

Chitosan and its derivatives, including glycolchitosan, were reported to inhibit fungal growth and to induce host-defense responses in plants and harvested commodities (4, 185). Combining 0.2% glycolchitosan with the antagonist *C. saitoana* was more effective in controlling green mold of oranges and lemons, caused by *P. digitatum*, and gray and blue molds of apples than either treatment alone (56, 57). Pretreatment of lemons with sodium bicarbonate further increased control of green mold on the light-green and yellow lemons (57). Other fruit coatings may also be useful for further reducing decay when applied with biocontrol agents (11, 39). For example, a fruit coating containing sodium salts of carboxy-methyl-cellulose,

sucrose esters of fatty acids, and mixed sucro-glycerides and soap, commercialized under the name TAL Pro-long, reduced the spread of a range of postharvest decays of pome fruits (11). The mechanism of action of TAL-Pro-long is not fully understood, but it reduces ripening and extends the natural resistance to invasion by the pathogen in storage.

Irradiation of root vegetables such as carrots (129) or sweet potatoes (167) with UV-C (wavelength below 280 nm) induced resistance and reduced postharvest decays. Attempts to irradiate pome, stone, and citrus fruit with UV-C and combine irradiation with biological control were largely unsuccessful (185). This may be attributed to a narrow range of the optimum UV-C irradiation dose, which is specific to different fruits, and variations of the resistance response with fruit maturity and storage temperature (45). The physiology of root tissue is very different from that of fruit, with the response of roots to UV-C irradiation more consistent (129, 167). This suggests that combining UV-C and biocontrol treatments may have a greater effect in controlling postharvest decays on roots than on fruits, and the role of the UV-C on fruits will be restricted mainly to its phytosanitary effect of reducing the survival of pathogen propagules.

GRAS substances such as sodium carbonate, sodium bicarbonate, and ethanol reduced conidial germination of *P. digitatum*, the causal agent of green mold of citrus (157, 158). Combining treatments of 3% sodium carbonate and the antagonist *P. syringae* ESC-10 was superior to either treatment alone in controlling green mold on citrus (159). This combination overcomes the significant shortcomings of both individual treatments. The antagonist alone is a poor eradicant and is usually incapable of controlling green mold on fruit inoculated with the pathogen 24 h before treatment with the antagonist. In contrast the carbonate salts control these infections (157). Carbonate salts, on the other hand, do not provide persistent protection from reinfection after treatment, whereas the antagonist persists for long periods after application and protects fruit from reinfection. Ethanol at 10%, in combination with ethanol-resistant *S. cerevisiae* strains 1440 and 1749, isolated from wine and ensile acorns, respectively, reduced the incidence of gray mold decay on apples from more than 90% to close to 0%, whereas either treatment alone did not reduce decay (122). The same concentration of ethanol reduced green mold of lemons to less than 5% (158). It will be interesting to determine the effect of ethanol in combination with an ethanol-resistant biocontrol agent on green mold of lemon.

Some non-fungicidal methods for control of postharvest diseases have been used on a small scale for some time and should be easy to combine with biocontrol treatments. Examples include sodium carbonate treatment of lemons, the addition of $CaCl_2$ to handling suspensions, and UV irradiation of pome fruit. Other approaches described above await implementation. Nevertheless, combinations of these treatments with biocontrol show great potential for enhancing control of postharvest decays of fruits.

Biocontrol product performance can be enhanced by applying the antagonist in a cascade similar to the hurdle technology strategy used in the food industry.

By incorporating various control steps along the packing line from receiving to packing, different combinations of products can be tested that more specifically suit individual packinghouses. For instance, by first drenching or fine-spraying fruit with a disinfectant such as chlorine, followed by hot water dip or ethanol spray, hot air drying, and finally biocontrol treatment incorporated into wax, effective control of mango, citrus, and avocado postharvest diseases can be provided. Creating a vacuum on the fruit surface and subsequently filling it with natural antagonists will not only reduce competition with other epiphytes, but will also stimulate the plant's natural defense system.

DEVELOPING THE COMMERCIAL PRODUCT

Biological control of plant diseases in general and on fruit after harvest in particular is a niche market, with a relatively small profit potential. Thus, finding an industrial partner has been the first challenge to public-sector researchers seeking to commercialize a biocontrol strain. In the case of BioSave development, the effectiveness of the antagonist, a saprophytic strain of *P. syringae* L-59-66, in reducing blue mold and gray mold decay on apples and pears in a laboratory setting was demonstrated to EcoScience Corp. (Orlando, FL). Then, large-scale feasibility tests were conducted in cooperation with the company. In these tests, the antagonist was applied to fruit on a commercial packing line, and after 3 months of storage, the fruit was evaluated for the development of decay. The commercial setting of the test, involvement of industry in conducting those tests, and encouraging results were the key factors in obtaining a commitment to develop the antagonist for commercial use (W.J. Janisiewicz, unpublished data). EcoScience Corp. investigated the potential for registration and formulation of the antagonist before making this commitment. Mass production by fermentation and the biomass yield of *P. syringae* strain L-59-66 was determined before scale-up experiments. The following years were focused on determining the spectrum of activity (92; W.J. Janisiewicz, unpublished data), testing the formulations developed by EcoScience Corp., testing the final formulation in a pilot test (90), and the development by EcoScience of safety data for registration of the antagonist. To build confidence in the product within the fruit industry, pilot tests were conducted in commercial packinghouses (99, 166). Extensive technical support and quality control have been instrumental in the success of this product. BioSave use has been increasing steadily over the past five years; during the 2000–2001 storage season, over 34 million bushel boxes of produce were treated with BioSave.

The commercial development of Aspire by Ecogen-Israel Partnership, Ltd. focused on the biocontrol of postharvest decays of citrus, mainly blue mold and green mold caused by *P. itallicum* and *P. digitatum*, respectively, which invade through wounds after harvest. The early research (29, 184) and the pilot test (48) in commercial packinghouses were conducted with a yeast biocontrol agent,

P. guilliermondii (originally described as *Debaromyces hansenii*) (126). Results indicated that a combination of the yeast with a tenfold diluted commercial rate of thiabendazole (TBZ) provided control equal to the full-strength fungicide. Subsequently, the focus has been changed to the yeast antagonist *Candida oleophila* (130, 191), previously identified as *C. sake* (187). Tests in commercial citrus packinghouses on oranges and grapefruit in Israel indicated that, like *P. guilliermondii*, *C. oleophila* gave satisfactory control of green and blue mold and sour rot caused by *Geotrichum candidum* only in combination with tenfold diluted TBZ (47). Perhaps a more effective antagonist could have been found if more emphasis was put on preliminary and secondary screening at the initial stage of research, and on selection criteria for antagonist designated for commercial development. In addition, changing the biocontrol agent during commercialization may have had an effect on the final product, as less extensive studies with the new antagonist were conducted. It is necessary to take into account as many aspects of biocontrol prior to commercialization as possible; hasty commitment to biocontrol agents without appropriate testing and classification should be avoided. The citrus system seems less prone to biocontrol than the pome fruit system. Combining yeasts with other control methods (188) appears to be a sensible approach at this time, although the citrus system may benefit greatly from more rigorous selection and manipulation of the antagonists to obtain a strain with enhanced biocontrol activity.

The research and commercial development of YieldPlus for biocontrol of pome fruit decays seems to follow the previously described example; however, little has been reported about that product.

The development of Avogreen followed a slightly different path, as this antagonist is applied in the field for postharvest biocontrol. Avogreen is a commercial formulation of the biocontrol agent *B. subtilis* isolated from avocado phylloplane, which has been registered in South Africa for control of *Cercospora* spot and anthracnose of avocado. Growers were encouraged to first test the product on a limited scale and integrate it with existing copper sprays. Support was provided to calculate dosage and adjust the existing spray schedule, which took into account equipment, cultivars, age, and history of the orchard disease profile. Developing different formulations was required to address the different needs of growers in terms of mixing, integrating with existing chemicals, and application method, which varied between low- and high-pressure equipment.

Marketing biological control products requires specialized knowledge of the target plant disease, the biological control agent, integrated disease control practices, production and storage systems, and microbial ecosystems. The success of implementing biocontrol or integrated disease management systems will depend largely on product knowledge and a thorough understanding of the complexity of the disease and postharvest environment. Distributing both the product and the knowledge necessary for its successful use will be the only effective way to ensure long-term market acceptance. These requirements are often overlooked in commercialization of biocontrol agents.

FUTURE PROSPECTS

This review reported the success of BCPD under laboratory and commercial conditions. The fact that the commercial introduction of BCPD increased the level of familiarity with this method of control by packinghouse personnel, regulatory agencies, private investors, and the public is important in advancing the commercial use of biological control (37). In addition, continuous increases in the use of BioSave since its large-scale commercial introduction in 1996, without an incidence of failure, boost user confidence in commercial biological control of plant pathogens.

The success of BioSave indicates that current biological control practices can be cost effective in large packinghouse systems. However, the quantitative relationship between the populations of the antagonist and the resulting control necessitates the presence of high cell densities of the antagonist in product, thereby cutting profit margins. Furthermore, postharvest practices in the Central and Eastern United States, which differ from those used in the Pacific Northwest, result in using an antagonist suspension not as efficiently as in the Pacific Northwest. Therefore, use of BioSave may be too costly in those regions. Biocontrol must be adapted to practices in different regions.

The issue now is not if or when BCPD will be used, but how broad its use will be and how fast it will expand to different commodities. BCPD has its limitations, which may restrict its use under some circumstances, but many of those limitations may be effectively addressed; this method is amenable to manipulation, as indicated in the many examples presented above. It would be inappropriate to equate biological control with fungicidal treatment without considering the advantages and limitations of both methods, which often differ. Hastily designed experiments comparing the two methods, without giving consideration to those factors, should be avoided. Instead, focus should be on circumstances where currently developed biocontrol can be used effectively. Every effort should be made to expand its use by the various improvement methods described above, including new areas, e.g., control of foodborne pathogens (89, 115), where fungicides are ineffective.

Our current model of biological control of plant disease is based on knowledge of natural processes of the antagonist-pathogen interaction. Although this traditional model is credited with numerous successes, including BCPD, we should move to adapt aspects of biotechnology as a means to improve disease control with even safer and more effective methods. In the well-defined environment of a postharvest system, there are unique opportunities to use microorganisms as a delivery system. In the future, it may be possible to use only strains adapted to postharvest conditions and introduce genes for biocontrol activity as needed. Development of microbial strains, as in developing new cultivars adapted to our needs, may become common practice in the future (37).

The science and practice of BCPD is still in its infancy compared to fungicidal treatment or field biocontrol, but the progress made in this area during the past

decade and a half has been remarkable. If this pace continues, the use of BCPD will be greatly expanded in the future.

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